



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁴ : C12N 15/00, 9/12	A3	(11) International Publication Number: WO 89/ 06691 (43) International Publication Date: 27 July 1989 (27.07.89)
(21) International Application Number: PCT/US89/00127 (22) International Filing Date: 12 January 1989 (12.01.89) (31) Priority Application Number: 143,441 (32) Priority Date: 12 January 1988 (12.01.88) (33) Priority Country: US (71) Applicant: CETUS CORPORATION [US/US]; 1400 Fifty-Third Street, Emeryville, CA 94608 (US). (72) Inventors: GELFAND, David, H. ; 6208 Chelton Drive, Oakland, CA 94611 (US). STOFFEL, Susanne ; 935 Galvin Drive, El Cerrito, CA 94530 (US). LAWYER, Frances, C. ; 6641 Saroni Drive, Oakland, CA 94611 (US). SAIKI, Randall, K. ; 320-39th Street, Richmond, CA 94805 (US).		(74) Agent: HALLUIN, Albert, P.; Cetus Corporation, 1400 Fifty-Third Street, Emeryville, CA 94608 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent). Published <i>With international search report</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 16 November 1989 (16.11.89)
(54) Title: PURIFIED THERMOSTABLE ENZYME (57) Abstract Recombinant DNA sequences encoding a thermostable DNA polymerase from <i>Thermus aquaticus</i> can be used to produce a recombinant protein with a molecular weight of about 86,000-95,000 daltons. The thermostable recombinant enzyme can be used in a temperature-cycling chain reaction wherein at least one nucleic acid sequence is amplified in quantity from an existing sequence with the aid of selected primers and nucleotide triphosphates. The enzyme is preferably stored in a buffer containing non-ionic detergents that lends stability to the enzyme.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	ML	Mali
AU	Australia	FR	France	MR	Mauritania
BB	Barbados	GA	Gabon	MW	Malawi
BE	Belgium	GB	United Kingdom	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IT	Italy	RO	Romania
BJ	Benin	JP	Japan	SD	Sudan
BR	Brazil	KP	Democratic People's Republic of Korea	SE	Sweden
CF	Central African Republic	KR	Republic of Korea	SN	Senegal
CG	Congo	LJ	Liechtenstein	SU	Soviet Union
CH	Switzerland	LK	Sri Lanka	TD	Chad
CM	Cameroon	LU	Luxembourg	TG	Togo
DE	Germany, Federal Republic of	MC	Monaco	US	United States of America
DK	Denmark	MG	Madagascar		
ES	Spain				

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 89/00127

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁴: C 12 N 15/00, C 12 N 9/12

II. FIELDS SEARCHED

Minimum Documentation Searched⁷

Classification System

Classification Symbols

IPC⁴:

C 12 N, C 12 Q

Documentation Searched other than Minimum Documentation
to the extent that such Documents are included in the Fields Searched *

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

Category⁸ | Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹² | Relevant to Claim No. ¹³

X	Abstracts of the Annual Meeting of the American Society for Microbiology, vol. 75, 1975 (US) D. Edgar et al.: "Purification and characterization of a DNA polymerase from an extreme thermophile, <u>Thermus aquaticus</u> ", page 151, abstract K 26 see the abstract	-	9-14,16
Y	--		15,17-26
X	Chemical Abstracts, vol. 85, no. 21, 22 November 1976, (Columbus, Ohio, US), A. Chien et al.: "Deoxyribonucleic acid polymerase from the extreme thermophile <u>Thermus aquaticus</u> ", see page 180, abstract 155559t, & J. Bacteriol. 1976, 127(3), 1550-7 (cited in the application)		9-14
X	Chemical Abstracts, vol. 93, no. 5, 4 August 1980, (Columbus, Ohio, US), A.S. Kaledin et al.: "Isolation and properties of DNA polymerase from		9-14,16

* Special categories of cited documents: ¹⁰

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

25th September 1989

Date of Mailing of this International Search Report

27 OCT 1989

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorised Officer

T.K. WILLIS

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	extremal thermophylic bacteria Thermus aquaticus YT-1", see page 377, abstract 40169p & Biokhimiya (Moscow) 1980, 45(4), 644-51 (cited in the application)	
Y	--	15,17-26
X	Chemical Abstracts, vol. 98, no. 7, 14 February 1983, (Columbus, Ohio, US), A.S. Kaledin et al.: "Isolation and properties of DNA polymerase from the extremely thermophylic bacteria Thermus ruber", see page 298, abstract 49311q, & Biokhimiya (Moscow) 1982, 47(11), 1785-91	9-14,16
Y	--	15,17,26
Y	Chemical Abstracts, vol. 105, no. 1, 7 July 1986, (Columbus, Ohio, US), A.K. Frey et al.: "Recovery of beta- galactosidase by adsorption from unclarified Escherichia coli homogenate" see page 491, abstract 5024g & Eur. Congr. Biotechnol. 3rd, 1984, 1, 655-63	15,17-26
A	EP, A, 0237362 (CETUS CORP.) 16 September 1987 (cited in the application)	
A	Proc. Natl. Acad. Sci. USA, vol. 85, December 1988 M.A. Innis et al.: "DNA sequencing with Thermus aquaticus DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA", pages 9436-9440	
P,X	EP, A, 0258017 (CETUS) 2 March 1988, see page 27, line 29 - page 31, line 38; claims	9-26
P,X	Science, vol. 239, 29 January 1988, R.K. Saiki et al.: "Primer-directed- enzymatic amplification of DNA with a thermostable DNA polymerase" pages 487-491, see the whole article	9-14

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

Please see Form PCT/ISA/206 dated 14-07-1989

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.